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RESEARCH NOTE

A COMPARATIVE STUDY OF SEXUAL BEHAVIOR IN TWO SYNMORPHIC SPECIES OF THE GENUS *LYCOSA* (ARANEAE, LYCOSIDAE) AND THEIR HYBRID PROGENY

Keywords: Spider hybrid courtship, hybrids, species-specific recognition

Lycosa thorelli Keyserling 1877 and *Lycosa carbonelli* Costa & Capocasale 1984 are synmorphic, synchronic and sympatric species (Costa & Capocasale 1984) that, however, can be collected in different microhabitats (unpubl. data). Males are slightly different in their female-searching behavior, but differ clearly in their courtship in front of females, thus avoiding interbreeding (Costa & Capocasale 1984). Costa & Francescoli (1991), using anaesthetized females, obtained an exceptional hybrid brood (from *L. thorelli* male and *L. carbonelli* female) and two conspecific control broods. These three broods were raised to adulthood (Francescoli & Costa 1992) and then used in the present study.

Costa et al. (1997), analyzing the behavior of parental (*L. thorelli* and *L. carbonelli*) and hybrid males elicited by female hybrid pheromone, found that hybrid males showed an intermediate behavioral pattern between both parental species and a low activity level. Parental males showed an intermediate activity level when compared to the activity elicited by conspecific and heterospecific stimuli.

In this paper, we analyzed (1) the behavior of the above mentioned males elicited by the parental species' sexual pheromone, and (2) the direct interactions among the males and the females of the three groups. This study allows a thorough comparison of the sexual behavior of the three groups released by different sexual stimuli. Also, these data facilitate both a deeper analysis of the function of reproductive isolation mechanisms and the formulation of hypotheses about signal effectiveness, mechanisms of heritability, and the possible evolutionary paths taken by the courtship behavior of these species.

We used 5♂ and 2♀ *L. thorelli*, 8♂ and 3♀ *L. carbonelli*, and 9♂ and 8♀ hybrids. Also, one wild-caught female *L. thorelli* and one wild-caught *L. carbonelli* were used as sex pheromone donors. Spiders were housed in the same conditions as in Costa et al. (1997). Voucher specimens were deposited in the entomological collection of the Facultad de Ciencias, Montevideo.

During the experiments the females were kept in glass containers (15 cm diameter × 5 cm high) with sand as a substrate. Two types of experiments were done. Males were observed in: (1) the presence of one parental sex pheromone, and (2) in the presence of both the female spider and the corresponding sex pheromone. In the first type, the females remained in the containers at least 24 h and were taken out immediately before the introduction of the male. The male was gently introduced, and his behavior was observed for 5 minutes. In the second type, we introduced a female at least 24 h before the experiment and the behavior of the male was then recorded after visual or tactile contact with the female. To reduce the probability of attacks, the male was introduced behind an opaque barrier. Experiments were ended when: (1) the male performed 60 minutes of sexual activity without copulation; (2) no sexual behavior was observed for 20 minutes; (3) copulation was completed; (4) the female attacked the male. Room temperature during the experiments was 23.4 ± 1.5 °C.

Forty-three trials were done, 15 in the context of female sex pheromone only and 28 in the context of the female and sex pheromone. The trials with pheromone only were (H = hybrids, C = *L. carbonelli*, T = *L. thorelli*; the first letter corresponds to the male and the second to the female): HC (4 trials), CC (2), TC (2), CT (2), TT (2), HT (3); data from HH, CH and TH were taken from Costa et al. (1997). The trials with females were: HH (5 trials), HC (3), HT (3), CH (3), CC (3), CT

Table 1.—Single records of rhythms and angles for leg movements. Data taken only from videos that allowed clear, on-screen measurements. Rhythms measured in movements/second; angles covered by leg movements measured in degrees. Groups were identified with two letters, the first corresponding to the male species and the second corresponding to the female (or pheromone donor): H = hybrids, C = *L. carbonelli,* T = *L. thorelli.* "—" denotes no data.

	Experimental group			
Behavior	HC	CC	CT	TT
Leg waving				
Rhythm	8.2	3.6	3.5	—
Angle	13.8	22.9	21.3	—
Rubbing				
Rhythm	—	—	—	11.8
Drumming				
Rhythm	7.0	4.0	5.0	4.6
Leg tapping				
Rhythm	—	2.7	3.3	—
Angle	—	28.0	25.1	—

(2), TH (3), TC (3) and TT (3). Individuals were used randomly, avoiding consecutive trials for the same individual. The low number of observations was because of the extremely limited number of available individuals (Francescoli & Costa 1992) and the risk involved in direct sexual encounters. The trials were video-taped and analyzed using 19 behaviors. Some behaviors are composed by more than one act that occur simultaneously. The behaviors observed in this study were: Abdominal vibrations (AV), Attack (At), Copulation (Co), Drumming (Dr), Explosive locomotion (EL), Immobility (Im), Leg tapping (LT), Leg waving (LW), Locomotion (Lo), Locomotion-with-Drumming (Lo/Dr), Locomotion-with-Leg tapping (Lo/LT), Locomotion-with-Leg waving (Lo/LW), Locomotion-with-Leg waving-with-Drumming (Lo/LW/Dr), Locomotion-with-Palpation (Lo/Pa), Locomotion-with-Palpation-with-Leg tapping (Lo/Pa/LT), Palpation (Pa), Positioning (Po), Rest posture (RP) and Rubbing of legs (Ru).

Comparisons using data obtained here and data from Costa et al. (1997) were made. The mean repertoire size comparisons used both sexual behaviors and all behaviors. Mean repertoire size was the average number of different behaviors presented in any experiment for each group. Single measurements of rhythms and angles for leg movements in some behaviors were obtained (Table 1).

Table 2.—Repertoire size in experimental groups responding to parental pheromone. "—" denotes no data. HH, CH and TH data were taken from Costa et al. (1997).

Group	Repertoire size	Number of observations	Repertoire size values as $\bar{X}$ (SD) All units	Sexual units
HH	21	46	5.80 (3.59)	3.78 (3.19)
CH	19	16	7.90 (3.20)	5.69 (3.28)
TH	22	15	8.13 (5.59)	6.07 (5.26)
HC	6	4	2.75 (2.22)	1.00 (2.00)
CC	6	2	3.00 (1.41)	2.00 (0.00)
TC	1	2	1.00 (0.00)	—
HT	5	3	2.33 (1.53)	1.33 (1.53)
CT	8	2	5.00 (1.41)	2.00 (2.83)
TT	9	2	5.00 (4.24)	3.00 (4.24)

The repertoire sizes of males stimulated by parental sex pheromone were smaller in relation to those elicited by hybrid pheromone (Table 2). Comparisons of mean repertoire size for the same kind of male and for the same kind of pheromone were made. In the first comparisons, HH was significantly different than HT (t = 3.37, $0.01 > P > 0.001$) and than HC (t = 2.48, $0.02 > P > 0.01$), using all behaviors. Using sexual behaviors, HH was different from HT (t = 2.45, $0.02 > P > 0.01$) and from HC (t = 2.52, $0.02 > P > 0.01$). TH showed the biggest mean repertoire size and TC showed the smallest one (all behaviors; t = 4.94, $P < 0.001$). CH was significantly different than CC (all behaviors: t = 3.0, $0.01 > P > 0.001$; sexual behaviors: t = 4.5, $P < 0.001$).

In the second type of comparisons, HH was significantly different than CH (all behaviors: t = 2.19, $0.05 > P > 0.02$; sexual behaviors: t = 2.02, $0.05 > P > 0.02$). In the intraspecific trials, sexual behaviors such as Leg waving, Drumming and Rubbing (and Explosive locomotion in *L. thorelli* male) were usually performed. In the interspecific trials only *L. carbonelli* males performed some sexual behaviors. Hybrid males performed Leg waving, Drumming, Palpation and Leg tapping as sexual behaviors in the presence of parental sexual pheromones.

Table 3.—Some behaviors observed in direct male-female encounters. Only presence of each unit in the experiences are listed. No sex = absence of sexual behavior. Experimental groups identified as in Table 1. Behavior abbreviations are LW = leg waving, Ru = rubbing of legs, Dr = drumming, AV = abdominal vibrations, EL = explosive locomotion, RP = rest posture, At = attack. One experiment of the CT group was deleted due to the absence of sexual behavior during the 20 minute period.

Group		Male					Female				
(*n*)	No sex	LW	Ru	Dr	AV	EL	LW	Dr	RP	At	Copulation
HH (5)	0	4	2	4	1	0	0	0	0	4	0
HC (3)	2	1	1	1	1	0	1	1	1	2	0
HT (3)	1	2	1	1	0	0	1	0	1	2	0
CH (3)	2	0	0	1	0	0	0	0	0	2	0
CC (3)	0	3	1	3	1	0	1	1	1	2	1
CT (2)	2	0	0	0	0	0	0	0	0	1	0
TH (3)	1	1	0	1	0	0	0	0	0	2	0
TC (3)	1	0	0	2	0	0	0	0	0	2	0
TT (3)	0	3	0	1	1	3	0	0	0	1	1

Data for male-female encounters are showed in Table 3. In one HC experiment, the male performed four unsuccessful mount atttempts.

Our results suggest that Leg waving, Drumming and Rubbing may be essential visual and acoustic signals for species recognition. These behaviors had constant species-typical characteristics (rhythms and angles) even when exposed to different pheromones. Parental females would discriminate slight differences in movement frequencies and angles from the signalling males. In *Lycosa malitiosa* Tullgren 1905, for example, the males were not recognized by conspecific females when the sexual signalling frequencies were experimentally changed (Costa & Sotelo 1983). Taking into account the complete precopulatory isolation between *L. thorelli* and *L. carbonelli* (Costa & Capocasale 1984), the absence of recognition of hybrid males by parental females was expected. However, the intermediate characteristic of the hybrid male signals elicited less intense rejections by parental females than the heterospecific males.

In the present study males showed a narrower repertoire than when exposed to hybrid pheromone (Costa et al. 1997) in both sexual and all behaviors. The absence of palpation in all its forms in males exposed to parental pheromones is remarkable, because of its occurrence in the presence of hybrid pheromone (Costa et al. 1997). This fact could be explained assuming that the hybrid pheromone is composed of species-specific tactochemical elements from both parental species, then increasing the male repertoires. In agreement with Costa & Capocasale (1984), *L. carbonelli* and *L. thorelli* males showed a poor repertoire when tested with the heterospecific pheromone. The absence of reaction in males in the two TC cases also supports this view.

In direct male-female encounters, male *L. carbonelli* were best at discriminating, because they displayed low sexual activity in response to *L. thorelli* and hybrid females (Table 3). This is in agreement with the results reported by Costa & Francescoli (1991) using anesthetized females. Hybrid males were the least discriminating, but they did not succeed in obtaining copulation.

The low attack level in female *L. thorelli* could be considered as indicative of sexual receptivity. The low level of sexual displaying in female *L. thorelli* does not indicate non-receptivity because these females are usually passive (Costa & Capocasale 1984). Our results show the absence of receptivity in hybrid females tested with the three types of males, and in parental females tested with heterospecific males. Stratton & Uetz (1986) reported similar responses in hybrid females of *Schizocosa ocreata* and *S. rovneri,* and rejection of hybrid males by parental females. The moderate tolerance of parental females to hybrid males would be based on the presence of some elements from both parental courtship behaviors.

The occurrence of copulations in conspecific experimental groups indicates that the laboratory conditions did not affect sexual communication. Thus, the absence of copu-

lations in the other groups suggests that natural hybrids—if they occur—will not reproduce. However, a *L. carbonelli* female received an intense courtship and repeated mounting attempts from one hybrid male. This female was receptive probably due to the recognition of some species-specific signals; but, at the mounting attempt, she could have detected chemotactile information from the males' integument, allowing rejection.

The characteristics of both parental species' courtship displays agree with the hypothesis from Bristowe & Locket (1926) on the origin of those displays by ritualization of searching movements. Furthermore, both species show similar behaviors when exposed to sex pheromone, but in presence of conspecific females, *L. thorelli* males change their behavior while *L. carbonelli* males maintain the searching pattern (Costa & Capocasale 1984). The common ancestor would have had a similar pattern to that of *L. carbonelli* because the pattern is performed in the searching phase by both species.

Although sympatric, *L. thorelli* are captured mainly in sunny short-grass areas, whereas *L. carbonelli* are captured in tall-grass areas, including dark and humid places. The Explosive locomotion performed by a *L. thorelli* male would only be seen in open areas. *L. carbonelli* shows a pattern fitted to dark and closed areas with multiple obstacles, consisting of "cautious" locomotion, and a high occurrence of Leg waving using their long legs. These two different habitats may have determined the distinctive characteristics of the observed courtship patterns.

The precopulatory isolation between *L. thorelli* and *L. carbonelli* could have evolved by a process of alteration in the communication codes, from a mutation or recombination of the genes responsible of the signalling frequency. Indeed, movement frequencies during some displays were greater in *L. thorelli* than *L. carbonelli* (Costa et al. 1997). Also, Explosive locomotion could have originated by a Lo/LW frequency increase alternating with prolonged immobility periods. In this process the well-known high selectivity level of the female should play the main role (Suwa 1985). Stratton & Uetz (1986) suggested that *Schizocosa ocreata* and *S. rovneri* speciated by alterations in the courtship pattern of their ancestor. In those species these authors postulated a model involving a mutation in "single autosomal loci." Results from *L. thorelli* and *L. carbonelli* suggest that more complex genetic determination mechanisms are involved.

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Fernando G. Costa: Etología, División Zoología Experimental, IIBCE. Av. Italia 3318, Montevideo, Uruguay

Carmen Viera and **Gabriel Francescoli:** Sección Entomología and Etología, Facultad de Ciencias. Iguá 4225, Montevideo 11400, Uruguay

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